NUCLEAR DIVISION OF SPIROGYRA

II. NUCLEAR DIVISION IN S. BELLIS

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(WITH PLATES XVIII–XX)

In investigating the nuclear division of *Spirogyra* three modes of procedure were used. Study of whole mounts fixed and stained gives a better idea of disposition of chromatic material, whereas a study of sections gives a better idea of its structure. For estimating duration of phases in karyokinesis and relative activity of parts of nucleus and boundaries of nucleus and cytoplasm, it is necessary to study living nuclei.

Although many species have been worked upon, none has yet been found that presents favorable material for all these methods. If nuclei are large, as in *S. crassa*, and so adapted for dissection from whole mounts or for sectioning, then in a living cell close winding of the chromatophores prevents a view of the nucleus in karyokinesis. Hence the study of *S. crassa* (8) was confined to the study of sections and entire nuclei dissected from the threads. In the studies of *S. bellis* a correlation was attempted of nuclear division stage by stage as seen in living cells with similar stages fixed the same evening. The nucleus of this species is plainly visible when living, and when stained its density is not so great but that its structures may be seen readily without sectioning. The stains used were safranin and gentian violet; Haidenhain's hematoxylin with iron alum; and anilin blue with eosin. Since gentian violet stains the cell sheath as well as the chromatic material, the last two combinations of stains gave the best results.

Material in good condition was obtained from three different sources. It is of interest that in these materials from widely separated localities uniform variations existed, yet all can be included under one type, a type differing markedly from that of *S. crassa*, already published, and from that of *S. dubia* and *S. ternata* now in preparation. In regard to the nuclear division of *S. dubia*, the discovery of 5 chromosomes was made, while in *S.*

ternata 4 chromosomes were found. This would seem to be the lowest number yet found in plants. The following quotation from DIGBY'S (3) investigation on *Crepis virens* states: "*Crepis virens*, as has been shown by ROSENBERG, possesses only 6 somatic chromosomes, the lowest number hitherto recorded in plants." Six chromosomes are also recorded by WISSELINGH (12) in *S. setiformis*, and by KARSTEN (5) in *S. jugalis*. The number of chromosomes in *S. bellis* agrees with that found by the writer (8) in *S. crassa*, namely, 14. Since a single genus of plants, as shown in *Spirogyra*, exhibits such striking differences in the number of chromosomes, there is no foundation for the assumption that nuclei in plants of a similar genus will show similar morphological organization. Morphological variation in the nucleus obtains in different species, just as variations occur in the number and winding of chromatophores and in cell dimensions.

In the 3 varieties of *S. bellis* also minor variations were seen in karyokinesis. So far as known to the writer, such variations in nuclear division have not been worked out in detail and established for any particular plant. MITZGEVITSCH (10), working on *S. subaequa* and *S. jugalis*, found differences in the distribution and origin of chromatic material in the prophase; but to establish distinctions in nuclear division, varieties in species as well as many species for comparison should be worked upon.

The dimensions of the vegetative cells of these 3 varieties of *S. bellis* Hass., and conforming to *S. bellis* as described by COLLINS (2), are as follows:

Var. *A*.—Vegetative filaments 57 μ in diameter, 95 μ in length, 3 or 4 spirals in the filament. This was gathered from the margin of a brook in which was intermingled *S. longata*, *S. inflata*, *S. gracilis*, at Northfield, Massachusetts.

Var. *B*.—Vegetative filaments 64 μ in diameter, 220 μ in length, 5 spirals in the chromatophore, the latter markedly dentate; border of river in Needham, Massachusetts. This was not a pure culture of *Spirogyra*, but intermingled were various filamentous desmids and *Mougeotia*.

Var. *C*.—Vegetative filaments 60 μ in diameter, 220 μ in length, uniformly longer than *B* and much longer than *A;* some threads

with 4 spirals, others with 5; fertile cells not swollen, zygospore oval. It also resembles *S. majuscula* Wolle. This material grew in pure culture in a swamp in a low-lying pasture.

Preparations of the 3 varieties show essentially the same structures. *C* was noteworthy for great variation in number and size of nucleoli. The 3 varieties show striking uniformity in formation and appearance of chromatic disks in approximately the same numbers. It was found advisable to make sketches of nuclei not at equal intervals of time, but at those periods when changes in the form and density of the component parts were more manifest. As some of these changes took place with great rapidity, others only appearing as a slow evolution, many rough sketches were made, not only following one nucleus throughout its changes, but also in different nuclei, studying particular phases repeatedly where changes were most rapid, to confirm interpretations made upon one. This seemed necessary, as many of the appearances were at variance with published results of other investigators. The rapidity of the changes in the living material explains why we get such variation in the fixed material.

From the appearance of the first change in the nucleus until the close of the reconstruction of the daughter nuclei, about an hour elapses; in some cases, 80 minutes. As the phases merge into one another, it is difficult to give precise time, but the following schedule may be taken as a typical example: prophase 15 min., metaphase 5 min., early anaphase 15 min., late anaphase and telophase 30–45 min. The changes in the nuclei are most marked and rapid in the first 3 phases. In late anaphase and telophase, although changes in translucency and shape occur, they do not result in such great differences in position; hence frequent sketches with constant comparison of observations were necessary to be assured of these changes. Since the structural organization of the nucleus is but the result of fixation of colloidal materials seen in living cells, effort was made to find in the living cells the homologue of the stained structure.

The quiescent nucleus contains a central spherical body (fig. 1), or more often in variety *C* two or more such bodies. This body may appear bluish or slightly opalescent as compared with the

more translucent rim that extends into the suspensors. It may be subtended by a similar appearing but crescent-shaped mass (fig. 2) likewise surrounded by a translucent rim. In fixed material the central body of the quiescent nucleus shows still greater variations in appearance. One or more vacuolar-like appearances may be seen within, or these may be lacking, the space being filled with substances differentiated in the double staining. Sometimes the central body may be composed of many small granules uniformly deeply stained, although double staining in the cell has differentiated parts of the chromatophore.

That such a body, generally termed the nucleolus, is a nucleolus in every species of *Spirogyra* appears very doubtful to the writer. The position and relative amount of cytoplasm suspending the nucleus and forming a border to the same, the extent of nuclear plasm, nucleolus, and nucleolar vacuole exhibit great variations in the different species. Hence the suspicion arises that some of the discrepancies in results obtained by investigators such as TRÖNDLE (11) and ZACHARIAS (13), working on the microchemical reactions of the central body, may be due to the fact that in some species the central body is a nucleus, the considerable space about it being cytoplasm; while in other species only a narrow border of cytoplasm exists, the nucleus with the contained nucleolus comprising all. The limits of this paper will not permit the extended comparisons of different species that would serve to establish such a view. The term central body will be used in preference to that of nucleolus, the main object here being to present certain new details in the history of the chromatic figure.

Prophase living

Although the central body appears to become diffused in enveloping plasm, as preliminary to this process an infusion of cytoplasmic substance flowing in through the suspensors must have taken place to account for the enlargement of the mass. This inflowing substance mixes with the nuclear plasm and denser substance in the spherical body, causing the sudden overflow as it were of the boundaries of the sphere. This change takes place with great rapidity, as shown by comparison of figures, beginning

with one drawn at 8:50 P.M., where a nucleus with its barriers still unbroken can be seen, the larger granules vibrating above and below, with the same nucleus drawn 10 minutes later, where the previously spherical body has become completely diffused in the enveloping plasm. While this change takes place, the merged mass of central body and enveloping plasm is no longer spherical, but exhibits amoeboid movements (figs. 3, 4, 14), the various parts appearing to change constantly in density. In some cases there seemed to be no streaming of the larger granules, the latter appearing either stationary or oscillatory. The overflow of nuclear content is but the manifestation of the disturbance of the ratio of nuclear mass to cytoplasm advanced by HERTWIG (4) as the cause of karyokinesis. This enlargement of the nucleus is coincident with the increase on all sides in the size of the suspensors (fig. 4). This appearance suggests that the suspensors act as the main channels, enlarged now as inlets for the superabundant assimilatory products derived from the chain of pyrenoids with which they are connected.

Finally, those suspensors which attach the nucleus to the chromatophores in the long axis of the cell appear to enlarge more rapidly than the others, showing that the main currents are now diverted in this direction. Meanwhile the large vesicles, gathered above the nucleus and appearing to aggregate there in a plane through the short axis of the cell, show active Brownian movements.

During this constant amoeboid movement of the more or less spherical mass, the turbidity which before extended through the mass to the bordering translucent rim now appears to clear in certain regions (fig. 5). It is to be noted, however, that in this turbid mass there is no trace of organization. Regions where changes take place are indicated in the drawings, where turbidity is shown by the gray shading. Lessening of the turbidity is to be seen, not at the equator, but at the lighter areas at some distance and also in the regions near the bordering rim.

Meanwhile, this whirlpool-like activity shapes the spherical mass into that of an ellipsoid. The granular folds of protoplasm that were constituents of the suspensors, and that lay at the margin of the more homogeneous protoplasm enveloping the

central body, appear to be pushed back on either side by the interior expansion of the mass, finally to take up a position at the poles of the spindle (figs. 15, 16).

Prophase fixed

Since each preparation could exhibit only the particular phase of activity at the instant of fixation, and since this period averages 15 minutes, it would be difficult to find two series of precisely equivalent stages. The differences manifested upon fixation of the turbid spherical mass can best be explained by references to the figures. These all show with the enlargement of the nucleus the unequal increase of the suspensors as the spindle begins its evolution. In all of the cells the beginning of the formation of the cell plate is to be seen before the dissolution of the nuclear membrane. No attempt is made to present the figures in sequence, as there is no evidence as to whether one stage follows another or as to whether in other nuclei a different disposition of substance may not obtain at corresponding intervals of divisions.

The chromatic granules, as in fig. 28, may be scattered on the main suspensor as well as over the central mass of granules. All of the chromatic substance may be gathered in a sphere to one side of the enlarged nucleus. This substance is in the form of granules (fig. 27) or of filaments and granules (fig. 25). In fig. 26 chromatic granules are connected by a finer network and distributed all over the enlarged nucleus. In the center lighter granules may be seen, suggesting a decomposing nucleolus. Fig. 29 shows lighter stained granules equally distributed over the mass. Scattered among them are short filamentous bodies. Finer granular masses in the center indicate the remains of a nucleolus. In figs. 31 and 33 is seen a somewhat contracted spherical mass, evidently both nucleolus and nuclear plasm, and consisting of granules both lightly and deeply stained. These are connected with the nuclear membrane by delicately stained strands. This and fig. 25 might be considered as a stage in synapsis. In fig. 24 no finer granular material is seen within the nuclear membrane, but there are chromatic masses ranging from tetrahedral forms to that of vesicles. Fig. 35 shows similar chromatic masses imbedded in or

overlying a less deeply stained material. Other figures show minor variations from those previously mentioned. In comparison, all that these stages appear to have in common is the increasing tendency of the material, chromatic and non-chromatic, to be peripherally arranged on a sphere.

Metaphase living

As the enveloping protoplasm gradually becomes more tenuous, adding substance to the retreating folds, the ellipsoid mass changes and becomes cylindrical. This appears to be due to the accumulation of denser portions on the surface and the gradual penetration of more liquid material to the interior. This may be forced out at the poles as the denser materials assume the form of a disk (figs. 5, 15, 16). The cylinder as it elongates loses progressively its turbid appearance. As the turbidity diminishes, the equatorial part retains the gray tint, forming gradually a dark band (fig. 17), while light bands by degrees evolve, encompassing the cylinder one on either side of the equator (figs. 16, 18). These bands as they assume concrete form acquire a translucent appearance. They condense into two disks connected by fragments of cords of a similar translucent appearance. It is to be seen, therefore, that instead of a sharp splitting and consequent clear-cut separation of equatorial masses of the cylinder occurring, no actual narrow rift was perceptible, although many nuclei at this stage were closely scrutinized for the expected splitting. There is no evidence in living material of these two disks having arisen from the splitting of discrete chromatic bodies. It is as if a gradual accumulation and rearrangement of materials had taken place, until finally the dense materials, appearing as jelly-like disks, are moving to the poles of the spindles, while vestiges of cords drag behind (figs. 19, 20).

Metaphase fixed

Figs. 34 and 35 represent transitional stages where the nuclear membrane has dissolved. Here the mass of stained material is beginning to lose its spherical shape; and the suspensors change, some in the long axis of the cell increasing and appearing as lines of granules directed, not as before, away from the margin of the

nucleus, but from the darkly stained mass. Around each of the deeply stained bodies a faintly defined areola can be seen (fig. 36). In subsequent stages, instead of being disposed without order on the periphery of the mass, they seem rather to form an equatorial band around the cylinder (figs. 37, 38). Those which seem to be well defined, and hence possibly properly called chromosomes, average 14 in number. They may become looped and arranged in such manner that ends of the loops may present an appearance as if constituting a single row of granules (fig. 40). The other granular material composing the cylinder now shows a tendency to longitudinal striation (figs. 41, 42, 43); later it takes the form of pyramids (figs. 46, 47, 48). When the masses take the form of pyramids the apices of the pyramids point to the poles of the spindle. The edges of the pyramids may stain almost as black with chromatin stains as the other bodies (figs. 46, 47). Comparison of living materials with stages showing pyramidal arrangement of chromatic substance suggests that the pyramids, with their apices always pointing to the spindle poles, are but fixations of the streams emanating from the oppositely charged jelly-like opalescent disks. A spindle inclined by pressure shows, as the mass condenses, small thickenings of chromatin appearing at the edge of the substance (fig. 39); hence the thickenings are not confined to the denser filaments in the bands. Again, in many sections the pyramidal appearance of the chromatic material suggests in its orientation an incompletely formed spireme (fig. 47). At this stage the pyramidal masses discharge droplets of material from the edge of the equatorial band (figs. 47, 52).

A gradual amalgamation and condensation of the two substances next occurs. The looplike nature of the lighter stained material and the pyramidal appearance are brought out in figs. 46 and 47. Analyses of these figures show that as they amalgamate they form groups, each group consisting of 4 masses inclosing a vacuole. Such structure of groups is like that described for groups making up chromosomes in *Allium* (9). Comparison of fixed cytoplasm shows that granules in the cytoplasm often assume this form. Its frequent occurrence shows that we have not a splitting of materials; hence such appearances could play no important part in theories

as to the reduction of chromosomes. This is probably, as LILLIE (6) suggests in regard to tetrad groups of *Ascaris* described by BRAUER, a purely physical phenomenon, a grouping due to precipitation of oppositely charged colloidal masses. These groups in turn are connected with each other by strings of less dense substance. A side view of them gives the impression of short dark bodies subtended by loops of lighter substance. They may present the same pyramidal appearance as seen before the separation of the disks. Pressure on the cover glass on turning the disks to full polar view shows the disks to be of no appreciable depth, and to consist approximately of 4 rows of tetrads (figs. 56, 57). As the disks exhibit a tendency to be attracted to the poles they become cone-shaped, the apex pointing in the long axis of the cell.

A gradual pulling apart of the amalgamated material follows, until, as in figs. 53–57, two opposing disks are seen. The position of these disks, their shape, and consequent behavior in pulling apart, show them to be but the fixed and stained masses which constitute the jelly-like bands seen in living material evolving from the turbid mass. The turbid mass corresponds to the fixed spherical mass composed of irregularly disposed filaments or granules. These disks of material as seen in fixed specimens are not preceded by the splitting of chromosomes in prophase, as stated by MITZGEVITSCH (10), BERGHS (1), and others. It is the amalgamated masses just as in *S. crassa* (8), the amalgamated spireme that appears to be pulled apart, forming two opposing networks in which no distinction of material now is apparent, unless it be in the linin-like connections of the groups.

With the formation of these networks, the space between the two becomes very clear. All the granules making up the spindles are absent, the intervening space between the networks being crossed by a few strands (figs. 51, 52) which in later stages appear to become attenuated and then disappear. These strands are identical with the translucent cords which in living material connect the separating jelly-like disks. Studies of living material raise the question whether the matter in the disks may not move more often to the poles in strands or streams not cohering in a disk, but reassembling in the form of disks when the poles are reached.

The network as it passes to the poles is seen to be not a uniform disk, but to become concave (fig. 55), the concavity pointing to the poles, indicating the direction of the attractive force. In some cases the concavity appears as a cone of granular material on a base of deeply stained tetrahedral masses (fig. 58). Arrived at the poles the network now converges to a spherical shape (figs. 59, 60), the chromatic material tending to become peripheral in position (fig. 62). Irregular masses of it later may be discharged. Fig. 67 illustrates the intimate connection of chromatic substance with pyrenoids. Figs. 62–68 show that as many variations mark the beginning of telophase as were seen in prophase. The chromatic masses become reduced in size, while the nucleolus as a reserve body appears in their midst.

Since the increase in size of the nucleolus is correlative with the reduction in chromatic masses, it is probable that it is not to be considered a karyosome, but derived indirectly from them.

Anaphase living

Returning to the study of living material, we find that this can be correlated step by step with that seen in fixed material. The movement of denser portions of disks away from each other does not always occur at once, as strands of the translucent substance connect the separating masses and also strands soon form connecting the granular polar masses (figs. 7, 17–20). These dense strands show no evidence of being composed of homogeneous bodies arranged as strings of beads. This accords with the observations on living material made by LUNDEGARDH (7).

While the translucent disks that we may term *a* and *b* are evolving and gradually retreating from one another, a similar (in appearance) jelly-like substance *c* and *d* appears at either end of the cylinder as it assumes the typical spindle form (fig. 7). Disks are represented by light areas. These evidently are not directly derived from the disks, as they are of considerable size before the approach of the latter; also, the disks do not appear to diminish as the strands bordering the cylinder increase. The ends *c* and *d*, partly under the chromatophore, show the same optical density, and are translucent and opalescent, while all else is grayish. The

substances *c* and *d* are evolved about 5 minutes later than the first appearance of *a* and *b*. Upon examining many kinds of fixed material, stages were found, as shown in figs. 38, 53, and 55, where polar chromatic disks similarly appear in metaphase and anaphase. In some cases only chromatic granules, in place of disks, are present, reminding one of centrosomes (fig. 50).

These substances appearing at the ends of the spindle may have been originally either at the border of the turbid mass seen in prophase (fig. 5), or else may be the accumulations discharged from the disk in metaphase as separate droplets (see fig. 62, from fixed material). Whether of cytoplasmic or nuclear origin, they appear to be of the same consistency as the disks having similar indices of refraction. The ends of the spindle are now lost to view in aggregations of granular matter that appear identical with the earlier suspensors. As the disks *a* and *b* approach the poles, a border of similar material appears, connecting them with disks *c* and *d* (fig. 8). This results in two irregularly shaped figures inclined to the quadrilateral, the jelly-like substance on the rim, the interior grayish.

This blending takes place before the two gels in their retreat have reached the polar granular masses. The substance in *c* then, probably contributed by the cytoplasm, rejuvenates the chromatic substance, and with this blending the disks likewise lose strands of their material to the cytoplasm. The changes in form and translucence of all substances in this stage take place with great rapidity. This accounts for the great variability and amorphous appearance of the separating disks as seen in prepared slides. The many kaleidoscopic shiftings of these masses, as illustrated in figs. 8–11 and 20–22, result in the appearance of a nucleolus within the grayish interior of each quadrilateral, while the gel forms the rim or daughter nuclear plasm. As movements subside, a reversal of optical refraction ensues, the central body appearing to increase in density, while the nuclear plasm becomes optically more like the hyaline cytoplasm (figs. 12, 13).

The daughter nucleus has manifestly received accessions from the cytoplasm at two periods in the karyokinesis: (1) when the sphere enlarges and becomes turbid; and (2) when disk *c*, apparently

from the cytoplasmic material, blends with *a* from the equatorial disk. Since disk *c* with *a* plays an important part in the reconstruction of the nucleus, and only disk *a* seems to coincide with the amalgamated chromatic material seen on the slides, it would appear that *Spirogyra* lends support to the views of those who believe that the chromosomes are not the sole containers of hereditary substances. It cannot be said that the material of the mother nucleus is equally divided between the two daughter nuclei, for all of the material in the two nuclei is not from the mother nucleus; while again, some of the colloidal material of the mother nucleus passes into the cytoplasm before the two daughter nuclei are formed. These observations also suggest that karyokinesis is no longer to be considered merely as a process of division, but as a process made up of alternating phases of addition, combination, and withdrawal of protoplasmic substances from nuclear centers.

Summary

Instead of a spireme, as in *S. crassa*, a disk arises from material condensing within the mass of nuclear plasm and central body. This disk is discernible in both living and stained material.

No trace of organization is to be seen in the living disk, but fixed material shows it to arise from aggregations of variable appearance and staining qualities. These aggregations are not the chromosomes. The more deeply stained of these bodies arise from the nuclear plasm, the less deeply stained appear to come from the decomposing central body.

This sphere of aggregated material gradually changes in shape, becoming a cylinder. The more deeply stained masses become arranged upon it as an equatorial band. This band is homologous with the disk seen in living material. As the disk evolves, chromatic bodies, averaging 14 for this species, are to be seen on the band, while other irregular masses of chromatic material project as loops or pyramidal masses from its edge. These loops or masses represent material from nuclear plasm and central body that has partially amalgamated.

No rift appears in the living disk to indicate a sharp splitting of components, but instead the changes in appearance indicate a

thinning in the center, while parts reassemble at either pole. The chromatic bodies in the fixed disks appear as viscous masses that, as they amalgamate, elongate, while other disconnected chromatic masses are discharged into the cytoplasm as the disk separates into the halves passing to the poles.

The living disks may be seen sometimes to pass *en masse* to the poles, but more usually they divide their substance into a few continuous strands, to reassemble as disks at the poles of the anaphase. These strands cannot be identified as moving chromosomes, since no units can be discerned in them. As the disks approach the poles, they appear to blend with similar disks apparently evolved from cytoplasm.

Each daughter disk thus arising upon fixation consists of a series of about 4 rows of tetrahedral masses. In living material the same appears as a translucent rim surrounding a less dense interior. The translucent rim becomes the nuclear plasm, while the central body takes shape within the less dense interior.

Spirogyra, as exemplified in *S. bellis* and *S. crassa*, may be characterized as having chromatic substance of a polymorphous nature; in the one a disk, in the other a spireme. The nucleolus does not fragment directly into chromosomes, as upheld by so many investigators, but only contributes the less dense substance seen at metaphase, which eventually may be discharged or become partially amalgamated with the chromatin. Hence *Spirogyra*, as regards the constitution and behavior of its nucleolus, need not be placed in a different category from the remainder of the green algae or from that of higher plants.

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LITERATURE CITED

1. BERGHS, J., Le noyau et la cinèse chez le *Spirogyra*. La Cellule **23**:53–86. 1906.
2. COLLINS, F. S., The green algae of North America. Tufts College Studies **2**: no. 3. 1910.
3. DIGBY, L., A critical study of the cytology of *Crepis virens*. Archiv Zellforsch. **12**:——. *pls. 8–10*.

4. HERTWIG, R., Über Korrelation von Zell und Kerngrösse und ihre Bedeutung. Biol. Centralbl. **23**: 49–62, 108–119. 1903.
5. KARSTEN, G., Die Entwickelung der Zygoten von *Spirogyra jugalis*. Flora **99**: 1–11. *pl. 1*. 1908.
6. LILLIE, R. S., The physiology of cell division. Amer. Jour. Physiol. **15**: 46–84. 1905.
7. LUNDEGARDH, H., Die Kerntheilung bei höheren Organism nach Untersuchungen an lebenden Material. Jahrb. Wiss. Bot. **21**: 236–281. 1902.
8. MERRIMAN, M. L., Nuclear division in *Spirogyra crassa*. BOT. GAZ. **56**: 319–330. *pls. 11, 12*. 1913.
9. ———, Vegetative cell division in *Allium*. BOT. GAZ. **37**: 178–207. *pls. 11–13*. 1904.
10. MITZGEVITSCH, L., Über die Kerntheilung bei *Spirogyra*. Flora **75**: 81–124. 1898.
11. TRÖNDLE, A., Der Nukleolus von *Spirogyra* und die Chromosomen von höheren Pflanzen. Zeitschr. Bot. **4**: 721–747, *figs. 11*. 1912.
12. VAN WISSELINGH, C., Über Kerntheilung bei *Spirogyra*. Flora **86**: 353–377. 1900.
13. ZACHARIAS, E., Die chemische Beschaffenheit von Protoplasma und Zellkern. Prog. Rei. Bot. pp. 67–257. 1910.

EXPLANATION OF PLATES XVIII–XX

All figures, except those of living nuclei, were drawn with the Abbé camera lucida, Leitz objectives, and Zeiss compensating oculars; drawings reduced one-half.

FIGS. 1–13.—Living nuclei of var. *C:* figs. 1 and 2, quiescent nuclei; fig. 3, early prophase at 9:07 P.M.; fig. 4, same at 9:15 P.M.; fig. 5, at 9:20 P.M.; fig. 6, at 9:25 P.M.; fig. 7, at 9:32 P.M.; fig. 8, at 9:36 P.M.; fig. 9, at 9:40 P.M.; fig. 10, at 9:44 P.M.; fig. 11, at 9:48 P.M.; fig. 12, at 9:52 P.M.; fig. 13, at 9:55 P.M.

FIGS. 14–23.—Living nuclei of var. *A:* fig. 14, at 7:45 P.M.; fig. 15, at 7:50 P.M.; fig. 16, at 7:55 P.M.; fig. 17, at 8:00 P.M.; fig. 18, at 8:07 P.M.; fig. 19, at 8:10 P.M.; fig. 20, at 8:12 P.M.; fig. 21, at 8:25 P.M.; fig. 22, at 8:28 P.M.; fig. 23, at 8:33 P.M.

FIGS. 24–68.—From preparations fixed in chromo-acetic; figs. 36, 46–48, 51, 52, and 56×3000; all others ×1800.